A Type-C Virus in Human Rhabdomyosarcoma Cells After Inoculation into NIH Swiss Mice Treated with Antithymocyte Serum

(RNA-dependent DNA polymerase/group-specific antigen)

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ABSTRACT A type-C RNA virus has been isolated that replicates readily in human and other primate cells. It was obtained from a human rhabdomyosarcoma cell (RD) that had been serially transplanted in immunosuppressed NIH Swiss mice, a strain of mouse from which infectious type-C virus has not been isolated. Various other human tumor cells, similarly transplanted, remained free of overt type-C virus expression. The virus growing in the RD cells, AT-124, has a group-specific antigen and an RNA-dependent DNA polymerase immunologically related to murine type-C viruses, but a host range similar to that of the RD-114 virus. The new isolate is either a previously undescribed, endogenous type-C virus from NIH Swiss mice or a recombinant with both mouse and human type-C genetic information.

The RD cell line originally described by McAllister *et al.* (1) is a human rhabdomyosarcoma cell line that propagates readily in culture. When these tumor cells are inoculated into fetal cats *in utero*, tumors develop. From one of these tumors, a type-C virus (2) called RD-114 was isolated that was shown to have a group-specific (gs) antigen and an RNA-dependent DNA polymerase that differed immunologically from that of the type-C viruses previously isolated from cats (3–5). The suggestion was therefore made that this virus may have been derived from the human RD cells or from some recombinational event between genetic information in the RD cells and the cat host in which the cells grew. Recently, methods have become available that use immunosuppressed animals treated with antithymocyte serum (AT) and that make it possible for human tumor cells to grow in a heterologous species (6–8). The NIH Swiss mouse was chosen because, although it has gs antigen and viral information expressed (9, 10), it is a strain with relatively low incidence of leukemia and has never been reported to produce complete, infectious type-C virus.

RD cells proved to be transplantable into NIH Swiss mice treated with antithymocyte serum†, and the tumors that developed in these animals could then either be re-explanted into cell culture or directly passaged to additional mice. From the third transplant generation, a cell line called AT-124 was established that was found to contain a type-C virus. This virus has a host range like that of the RD-114 virus, i.e., it grows readily in various human and primate cells, but grows

poorly, if at all, in murine cells. However, the gs antigen and the RNA-dependent DNA polymerase of this virus are shown to be antigenically closely related to mouse type-C viruses. The possibility is discussed that this virus may be a recombinant with both mouse and human type-C genetic information. The alternative possibility is that it represents the isolation of the endogenous NIH Swiss type-C virus and that the RD cell serves as a permissive host for replication of this virus.

MATERIALS AND METHODS

Animal Inoculation. RD cells, obtained from Dr. Robert McAllister, Children's Hospital of Los Angeles, were inoculated into the subcutaneous tissue of 16-day-old NIH Swiss mice treated with repeated 0.15- to 0.25-ml doses of rabbit anti-(mouse thymocyte) serum (product 57-110) obtained from Microbiological Associates, Bethesda, Md. Each mouse received 2×10^6 viable tumor cells. Tumors developed subcutaneously and also adjacent to the spleen, apparently by extension through the abdominal wall. Tumors had a wide range of growth potential, but each of the tumors shown in Table 1 grew progressively and has given rise to a serially transplantable tumor. The RD cell tumor developed initially as a peritoneal tumor adjacent to the spleen. This tumor was subsequently transplanted two more times into AT-treated NIH Swiss mice. The third transplant generation was explanted and grown in cell culture in Dulbecco's modification of Eagle's medium with 10% calf serum. The explanted cells have the morphologic and karyotypic properties of human rhabdomyosarcoma cells†.

Viral Polymerase and Group-Specific Antigen Determinations. Supernatant fluids from the various human cell cultures that had been transplanted in NIH Swiss mice were concentrated 70-fold by centrifugation, assayed for viral RNA-dependent DNA polymerase, and tested with rabbit antisera prepared against the viral enzyme (11, 12). The use of complement fixation procedures and radioimmunoassays for detection of the major group-specific antigen of type-C viruses has been described (13).

Host Range of Virus Isolates. Various cells from different species that have been useful for studying the replication of known type-C viruses were tested. These include mouse cell lines BALB/3T3 (14) and NIH/3T3 (15), a rat cell line NRK (16), a cat-embryo cell strain (FEF) (17), kindly provided by Dr. P. Fischinger (Nat. Cancer Inst.), a continuous line of

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TABLE 1. *Particulate supernatant DNA polymerase in culture fluids of human tumor cell lines passed in immunosuppressed NIH Swiss mice*

Cell line	Tumor type	Source	Polymerase (pmol [³H]TMP incorp.)*
AT-124†	Embryonal rhabdomyosarcoma	(1)	27.3
AT-130	Carcinoma, colon	(§)	<0.5
AT-144	Carcinoma, cervix	(30)	<0.5
AT-151‡	Liposarcoma	(31)	<0.5
AT-155	Carcinoma, breast	(32)	<0.5
AT-161‡	Liposarcoma	(31)	<0.5
AT-166	Carcinoma, pharynx	(33)	<0.5
AT-189‡	Liposarcoma	(31)	<0.5
AT-203	Carcinoma, breast	(34)	<0.5
AT-210	Congenital spherocytosis	(35)	<0.5
AT-218	Carcinoma, cervix	(36)	<0.5

* Supernatants from cultures were concentrated 70-fold by centrifugation and assayed for DNA polymerase with poly(rA)·oligo(dT) as described, with 30 mM [³H]TTP (6000 cpm/pmol) (4, 11).

† McAllister's rhabdomyosarcoma RD, free of virus; AT-124 represents third transplant generation in AT-treated NIH Swiss mice.

‡ Morton's liposarcoma, passageable serially by direct transplant of tumors as with RD. AT-151 = transplant passage 1; AT-161 = transplant passage 2; AT-189 = transplant passage 3.

§ Arnstein, P., Nelson–Rees, W., Taylor, D. O., Huebner, R. J. & Lennette, E. H., in preparation.

rhesus monkey lung cells, DBS-F-RhL-1 (18), from Dr. Rosylyn Wallace (Lederle Laboratories, Pearl River, New York), and two human cell strains developed in this laboratory, 525T and A204 ‡. The former is a normal diploid fibroblast strain from adult skin and the latter is a human rhabdomyosarcoma cell line.

RESULTS

While at least eight other human tumors passaged in AT-treated NIH Swiss mice were negative for supernatant RNA polymerase, AT-124 cells were clearly positive, as indicated in Table 1. All of the cells, and, in particular, the original RD cell, that are described in Table 1 had also been tested for supernatant polymerase before animal inoculation and were negative. The supernatant polymerase from AT-124 cells was tested with rabbit antisera prepared against the known RNA-dependent DNA polymerase of mammalian type-C virus, including mouse, cat, primate, and RD-114 viruses (4, 12). As shown in Table 2, the only antiserum to inhibit the RNA-dependent DNA polymerase activity was that prepared against the mouse type-C viral polymerase. Cell extracts were tested with the various antisera specific for the major viral internal protein, the gs antigen (19, 20), of the mouse, cat, primate, and the RD-114 viruses by complement fixation and radioimmunoassay (13). As shown in Table 3,

‡ Giard, D., Aaronson, S. A., Todaro, G. J., Kersey, J. H., Dosik, H. & Parks, W. P., *J. Nat. Cancer Inst.*, manuscript submitted.

TABLE 2. *Effect of rabbit antipolymerase IgG on enzyme activity*

Polymerase source	Serum added	pmol [³H]TMP incorp.*
AT-124	None	3.5
	Anti-pol (MuLV)	0.2
	Anti-pol (RD-114)	4.3
	Anti-pol (Woolly)	2.9
	Anti-pol (FeLV)	2.7
	Anti-pol (RSV)	3.9
MuLV	None	2.4
	Anti-pol (MuLV)	0.1
	Anti-pol (RD-114)	2.1
RD-114	None	4.3
	Anti-pol (MuLV)	4.8
	Anti-pol (RD-114)	0.5

* Assay was performed as described (4). In all cases the rabbit antibody used was the IgG fraction purified by DEAE-cellulose chromatography. Pol, polymerase; MuLV, murine leukemia virus; FeLV, feline leukemia virus; RSV, Rous sarcoma virus; woolly, woolly monkey type-C virus.

only mouse gs antigen could be detected in AT-124 cells. Electron microscopy of the AT-124 cells has also shown typical budding type-C viruses.

In order to determine the infectivity of the virus from the AT-124 mouse cells, BALB/c 3T3, NIH Swiss 3T3, as well as rat cells (NRK clone 2), primate cells (DBS-1), and human cells (525T and A204) were tested by inoculation of 5 × 10⁵ cells per flask with 2 mg/ml of polybrene and then infection with filtered supernatants from AT-124 cells. At 14 days, the supernatant polymerase was tested, and the results are shown in Table 4. The virus grows readily in the human, nonhuman primate, and feline cells tested and replicates to a lesser degree in NRK cells, but fails to show detectable replication in mouse cells. Although the virus has a mouse RNA-dependent DNA polymerase and a mouse gs antigen, primate and feline cells are its preferred hosts. So far, natural isolates of mouse type-C viruses have propagated either in NIH Swiss (N-tropic viruses) or in BALB/c cells (B-tropic viruses) (21, 22); this virus grows in neither.

Table 4 shows, further, that the host range of the AT-124 virus is comparable to that of the RD-114 virus. Neither RD-114 nor AT-124 virus replicates to a detectable degree in either of the mouse cell lines tested.

In a related set of experiments, the RD and other human tumors have been serially transplanted in AT-treated AKR mice†. This mouse strain with high leukemia incidence has infectious N-tropic virus that can be isolated from virtually any AKR tissue (22). The RD tumor cell line established after serial passage in AKR mice (AKR-AT-152) was found to contain virus, but, as shown in Table 4, the host range of the AKR-AT-152 virus is very different from that of the AT-124 virus. The former appears to be a typical AKR virus isolate, growing readily in NIH/3T3 and in NRK cells, but not in any of the three primate cells tested. This strongly suggests that merely growing a typical, infectious murine type-C virus in the RD cells does not, in itself, confer an altered host range on the isolate.

The AT-124 virus, by itself, has shown no evidence of transformation in any cell system so far tested; it is therefore

TABLE 3. *Detection of species-specific gs antigen in the AT-124 cell line* *

Assay for the major viral antigen	Concentration (ng/mg of protein)	CF titer
Murine	330	2
Feline	<2	<2
Woolly	<2	<2
RD-114	<2	<2

* Assays were performed as described (13, 10) with a 20% cell extract (v/v).

concluded that it is a helper or "leukemia" virus rather than a transforming or "sarcoma" virus. When AT-124 cells are cocultivated with "nonproducer" NRK cells transformed by murine sarcoma virus (23), the sarcoma virus is rescued, and the host range for focus-forming ability with the murine sarcoma virus (AT-124) pseudotype corresponds to the host range for infectivity of AT-124 itself. The murine sarcoma virus (AT-124) pseudotype readily produces transformed foci on human and on other primate cells§. The ability of AT-124 virus to rescue the sarcoma genome from the transformed NRK cells directly demonstrates its helper activity.

Since virus was isolated from the third transplant passage in AT-treated NIH Swiss mice, the question arose as to whether the virus could be detected in tissues or in tissue culture materials from earlier passages. The first transplant passage in NIH Swiss mice, AT-97, neither had detectable titers of type-C virus nor was the virus inducible from the explanted RD cells by the addition of thymidine analogs (24–26). To test the possibility that AT treatment alone is enough to allow virus expression in NIH Swiss mice, the spleens of several AT-treated mice as well as those that had been inoculated with various other human tumors were tested for virus. In each case the results were negative for whole virus production by the polymerase assay and for infectivity by testing on both rodent and on primate cells, including those found to be most susceptible for the growth of the AT-124 virus.

DISCUSSION

Two type-C viruses (RD-114 and AT-124) have now been isolated from the RD cells after heterotransplantation into immunologically-compromised animals. This paper reports the isolation and partial characterization of a virus in a human rhabdomyosarcoma cell that had been passaged through a strain of mouse (NIH Swiss) that is free of infectious type-C virus. The finding of a type-C virus that replicates to high titers in various nonmurine cells and that has murine RNA-dependent DNA polymerase, the murine type-C species-specific antigen, and the ability to rescue a sarcoma virus from nonproducer cells, indicates that between the NIH Swiss mouse and the RD tumor cell line there exists the potential to produce a complete, infectious type-C virus. The inability to isolate virus from other human tumors growing in similarly treated mice and the failure to find infectious virus in the spleens of chronically immunosuppressed NIH Swiss mice would suggest that the RD cell contributes

§ Todaro, G. J. & Meyer, C., in preparation.

TABLE 4. *Host range of the AT-124 virus*

Cell lines tested	[³H]TMP (pmol incorp.)*			
Mouse	AT-124	RD-114	R-MuLV	AKR-AT-152 (2)
NIH/3T3	<0.5	<0.5	85.2	74.2
BALB/3T3	<0.5	<0.5	57.0	<0.5
Rat				
NRK	8.3	15.2	6.3	124
Cat				
FEF	185	52.8	NT	NT
Primate				
DBS-F-RhL-1	38.2	75.2	<0.5	<0.5
Human				
525T	18.6	32.3	<0.5	<0.5
A204	86.8	192	<0.5	<0.5

* Supernatants from cultures exposed to virus taken 2 weeks after viral infection, concentrated 70-fold, and assayed for viral polymerase as described (11, 29).

some function that allows viral replication to be completed. Further studies are necessary to characterize the antigens and the nucleic acid of the AT-124 virus and to compare them with the RD-114 virus, the recently described primate type-C viruses (27, 28), and the endogenous type-C virus from mouse (25, 29) and cat¶ cell clones. Whether the AT-124 virus is able to grow readily in RD cells because of a contribution of endogenous type-C virus genetic information by the host cell or because the intracellular environment of the RD cell is permissive for replication of certain endogenous type-C viruses remains to be determined.

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